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Arterial blood gases, electrolytes, and metabolic indices associated with hemorrhagic shock: inter- and intrainbred rat strain variation

Rajiv Rose, Bijan S. Kheirabadi, and Harold G. Klemcke

U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas

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Rose R, Kheirabadi BS, Klemcke HG. Arterial blood gases, electrolytes, and metabolic indices associated with hemorrhagic shock: inter- and intrainbred rat strain variation. *J Appl Physiol* 114: 1165–1173, 2013. First published March 7, 2013; doi:10.1152/japplphysiol.01293.2012.—We have previously shown interstrain variation (indicating a genetic basis), and intrastrain variation in survival time after hemorrhage (STaH) among inbred rat strains. To assist in understanding physiological mechanisms associated with STaH, we analyzed various arterial blood measures (ABM; pH, Pa_{CO_2}, oxygen content, sodium, potassium, glucose, bicarbonate, base excess, total CO_2, and ionized calcium) in inbred rats. Rats from five inbred strains (n = 8–10/strain) were catheterized and, ~24 h later, subjected to a conscious, controlled, 47% hemorrhage. ABM were measured at the start (initial) and end (final) of hemorrhage. Inter- and intrainbred strain variations of ABM were quantified and compared, and correlations of ABM with STaH were determined. All final ABM values and some initial ABM values were different among strains. Most ABM changed (Δ) during hemorrhage, and these changes differed among strains ($P < 0.03$). Some strain-dependent correlations ($r \geq 0.7$; $P \leq 0.05$) existed between ΔABM and STaH (e.g., BN/Mcwi, ΔK^+, $r = -0.84$). Dark Agouti rats (longest STaH) had the smallest ΔPa_{CO_2}, ΔHCO_3^-, and Δbase excess, and the highest final glucose. High coefficients of variation (CVs, >10%), strain-specific CVs, and low intraclass correlation coefficients ($r_I < 0.5$) defined the large intrastrain ABM variation that exceeded interstrain variation for most ABM. These results suggest that some ABM (K^+, Pa_{CO_2}, glucose, oxygen content) could predict subsequent STaH in an inbred rat strain-dependent manner. We speculate that whereas genetic differences may be responsible for interstrain variation, individual-specific epigenetic processes (e.g., DNA methylation) may be partly responsible for both inter- and intrastrain ABM variation.

intrainbred strain variation; intraclass correlation coefficient; hemorrhage; arterial blood gases; Pa_{CO_2}; oxygen content; blood electrolytes

WE RECENTLY INITIATED a series of studies to identify genes that may regulate early survival after hemorrhagic shock (33–35). As we attempt to first identify quantitative trait loci (QTL) and, ultimately, one or more individual genes, we also make use of various phenotypic indicators that might identify important response mechanisms to severe hemorrhage. After acute, severe hemorrhage, multiple compensatory systems are activated in attempts to maintain homeostasis, especially to preserve perfusion of the brain and heart (11, 44). Arterial blood measures (ABM) associated with cellular responses to hemorrhagic shock reflect early cellular changes and compensatory responses. Such ABM include arterial blood gases (Pa_{O_2}, Pa_{CO_2}, oxygen content); electrolytes (potassium, sodium, calcium); and metabolic indices [base excess (BE), lactate] (19, 60). Many such measures have been shown to differ between those who survive and those who do not survive after similar hemorrhages in both humans (6, 61–62) and rats (67–68). In some cases, such measures (e.g., potassium level, negatively correlated to survival) have been suggested as predictors for an individual's ability to survive hemorrhagic shock (67–68).

In our initial set of studies using five inbred rat strains, we observed high interstrain and intrastrain variability in survival time after a 47% conscious, controlled hemorrhage (survival time after hemorrhage; STaH) (34). The current work examines *1)* differences in some ABM among the five inbred strains that might reflect cellular mechanisms associated with different STaH; *2)* correlations of these ABM with STaH; *3)* changes occurring in these ABM in the five inbred rat strains while transitioning from normovolemic to a hypovolemic state; *4)* intrastrain variation of ABM to highlight its presence and magnitude; and *5)* the possible sources of the inter- and intrastrain variation observed in ABM.

MATERIALS AND METHODS

Animals

All rats were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX. Animals received humane care in accordance with the *Guide for the Care and Use of Laboratory Animals* (7th edition, 1996). Five inbred rat strains were used for the study: *1)* Brown Norway Medical College of Wisconsin (BN/Mcwi), n = 9, body wt 297 ± 4 g (Medical College of Wisconsin, Milwaukee, WI); *2)* Fawn Hooded Hypertensive (FHH), n = 10, 315 ± 5 g (Physiogenix, Milwaukee, WI); *3)* Dahl Salt-Sensitive (SS), n = 9, 403 ± 10 g (Charles River Laboratories, Wilmington, MA); *4)* Dark Agouti (DA), n = 8, 262 ± 7 g; and *5)* Lewis (LEW), n = 9, 324 ± 8 g (Harlan, Indianapolis, IN). These five strains were found to be the most divergent in STaH, and have congenic and consomic strains, which are needed for expediting QTL analyses in future studies (34). All rats were males, shipped at ~10 wk of age, and were held in our vivarium for an 18- to 24-day acclimation period before experimentation. Rats were housed individually in plastic cages (27.3 × 48.9 × 27.3 cm) at 19 to 23°C room temperature, with lights on from 0600 to 1800 h. Food (Harlan Global Teklad 2018, Madison, WI) and water were constantly available. Relative humidity ranged between 55% and 65% during each 24-h period. Rats were randomly assigned to day of surgery, order of surgery on each day, and order of hemorrhage on each day.

The surgical procedures (i.e., catheterization) were conducted under aseptic conditions as described in detail previously (33). For surgical procedures, rats were initially anesthetized with 5% isoflurane in 100% oxygen (induction period) via face mask. The oxygen flow rate was 2.0 liters/min. After an induction period of 5 min, the isoflurane was reduced to 3% for ~10 min until surgery was underway, and then reduced to 2.5% and maintained at 2.5% until all aspects of the surgery were completed. An analgesic (buprenorphine 0.025 mg/kg sc) was administered at the end of surgery before termination of anesthesia to relieve postoperation pain. Approxi-

Address for reprint requests and other correspondence: H. G. Klemcke, U.S. Army Institute of Surgical Research, 3650 Chamber Pass, Fort Sam Houston, TX 78234 (e-mail: harold.klemcke@amedd.army.mil).

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mately 24 h after surgical catheterization, rats were subjected to conscious controlled hemorrhage wherein 47% of circulating blood volume was removed in two steps in a total of 26 min as described earlier (33–34). Average strain-specific blood volumes per 100 g body wt previously measured using Evans blue dye technique (34) were used along with body weight (on the day of surgery) to calculate the total blood volume per rat. This was then used to calculate the amount of blood to be removed from each rat for a 47% hemorrhage. After hemorrhage, rats were observed for a maximum of 4 h. Surviving animals were euthanized with an intravascular injection of sodium pentobarbital (150 mg/kg body wt) at 4 h posthemorrhage.

Arterial Blood Measures

Arterial blood gases, electrolytes, and metabolites were determined with the i-STAT System using the CG8+ Cartridge (Abbott Laboratories, Abbott Park, IL). i-Stat instrument standards containing low and high levels of each ABM were measured on three occasions (approximately at the beginning, middle, and end of the study) to check the accuracy of measurements. ABM were measured from 0.5-ml blood samples that were drawn at two time points; at the first (1st minute; initial; normovolemic) and last (26th minute; final; early hypovolemic) minute of hemorrhage. Changes in ABM (denoted as ΔABM) are the difference between the final and initial values for each ABM. Arterial blood measures of interest included pH, partial pressure of arterial carbon dioxide (Pa_{CO_2}), total carbon dioxide [TCO_2], oxygen content, sodium concentration [Na^+], potassium concentration [K^+], ionized calcium [Ca^{++}], bicarbonate concentration [HCO_3^-], glucose concentration [glucose], and [BE]. Total CO_2 was calculated as follows: TCO_2 (mmol/liter) = HCO_3^- + 0.03 Pa_{CO_2}.

Oxygen content was calculated as (45, 68) follows:

$$\text{Oxygen content (ml/dL)} = (\text{Hb} \times 1.34 \times Sa_{O_2}) + (0.003 \times Pa_{O_2})$$

where Pa_{O_2} = arterial oxygen pressure, and Sa_{O_2} = arterial oxygen saturation. Oxygen content reflects the total volume of oxygen, both bound and unbound, present per 100 ml of blood (41).

Experimental Design and Statistics

The study was designed to be a randomized, complete block experiment wherein rats from each strain would be equally represented within each week of experimentation. However, due to limitations in availability of rats, this optimal design was not achieved. Nonetheless, representatives of each strain were included throughout an 8-wk experimental period.

Data were analyzed using the Statistical Analysis System package version 9.2 (SAS, Cary, NC). Graph-Pad Prism version 5 (GraphPad Software, La Jolla, CA) was used to generate the scatter plots in the figures. Interstrain differences in ABM were analyzed using two-way ANOVA for repeated measures, whereas strain differences in ΔABM were analyzed using one-way ANOVA. Differences in individual means were examined using the a posteriori Student-Newman-Keuls test, or a multiple *t*-test adjusted for multiple comparisons via the false discovery rate (3). All data were tested for homogeneity of variance (Levene's test) and normality of distribution (Kolmogorov-Smirnov test) and, wherever necessary, data were transformed using log or square root functions. Correlation analyses were conducted using PROC CORR of the SAS system. Because correlation coefficients and their associated probability levels can be deceptive, all data were plotted (using PROC GPLOT), and the scatter of data points around the best-fit line were observed. Only those correlations associated with coefficients of determinations (r^2) ≥0.5 were considered potentially biologically relevant and presented (i.e., ≥50% of the variation in one variable is due to variation in the second variable). For some strains (especially DA), correlations with STaH involved use of censored data; that is, rats were euthanized at 240 min, and the true survival time could not be measured. Such correlations may not accurately reflect the true association with STaH.

Within-strain variability was examined by calculating coefficients of variation (CVs) and intraclass correlation coefficients, r_I. CVs were calculated as the standard deviation divided by the mean and reported as a percentage (16). CVs were statistically compared (77) among strains for individual measures (i.e., to determine whether an inbred rat strain exhibited more variability than others for a given measure). Intraclass correlation coefficients were determined by calculating the relative magnitude of the variance component among groups (s^2_A) and the error variance (s^2). The variance components are summed, and each variance component is expressed as a percentage of their sum. The percent of variation among groups divided by 100 gives r_I, which represents the proportion of the total variation that exists among groups (in our case, among inbred rat strains) (64). A high r_I (i.e., >0.5) indicates that interstrain variation is more than intrastrain variation. If $r_I = 1$ (maximum value), then there is no variation within strains [i.e., measures within groups (strains) are identical].

RESULTS

Differences in Blood Measures among Strains and Time Points

Survival times following hemorrhage for the inbred rat strains used in this study and their variability have been reported earlier (34). The initial values for some ABM (Pa_{CO_2}, TCO_2, O_2 content, Na^+, Ca^{++}, HCO_3^-) were different ($P < 0.05$) among the five inbred rat strains, whereas others (pH, K^+, glucose) were comparable (Figs. 1–3). Final values of all ABM were different among the five inbred strains (Figs. 1–3). For all inbred rat strains, each ABM, except Ca^{++}, changed during the course of the hemorrhage (Figs. 1–3, Table 1). Most ABM decreased during the 26-min bleed (Pa_{CO_2}, HCO_3^-, BE, Na^+, TCO_2, O_2 content), but a few increased (pH, K^+, glucose). Arterial blood pH was the least altered measure, with modest increases observed in only FHH and SS rats (Fig. 1*C*; Table 1). Moreover, these changes in ABM, with the exception of Ca^{++}, showed strain-dependent differences (Table 1).

Differences in Blood Measures Within Strains

The amount of intrainbred strain variation observed visually via scatter plots of STaH (34), and of the initial and final ABM (Figs. 1–3), were intriguing in light of the use of genetically similar inbred rat strains. Intrastrain variations in ABM were statistically analyzed via CVs and r_I.

Coefficient of variation. High CVs (>10%) were observed for some initial ABM such as BE, glucose, K^+, and for most final ABM such as Pa_{CO_2}, HCO_3^-, TCO_2, BE, K^+, and glucose (Table 2). Instrument standards were measured on 3 different days during the study. With the exception of standard BE values, other standard ABM values were remarkably constant throughout the study. The CVs of most ABM standards were considerably less than ABM in blood samples. The CVs for the low and high ABM standards, respectively, were as follows: Pa_{CO_2}, 2.6%, 4.0%; HCO_3^-, 2.9%, 2.8%; TCO_2, 2.4%, 2.3%; pH, 0.02%, 0.08%; BE, 10.8%, 21.6%; Na^+, 0.0%, 0.4%; K^+, 2.0%, 0.9%; glucose, 1.4%, 2.3%; Ca^{++}, 0.8%, 1.2%; Pa_{O_2}, 3.4%, 1.3%; oxygen saturation, 0.6%, 0.0%.

The CVs of some blood measures differed among the inbred rat strains at both the initial and final time points (Table 2). Interstrain comparisons of CVs between initial and final values of each ABM revealed greater variability in CVs of final pH, Pa_{CO_2}, HCO_3^-, and TCO_2 than their corresponding initial CVs (Table 2, *Final* vs. *Initial*).

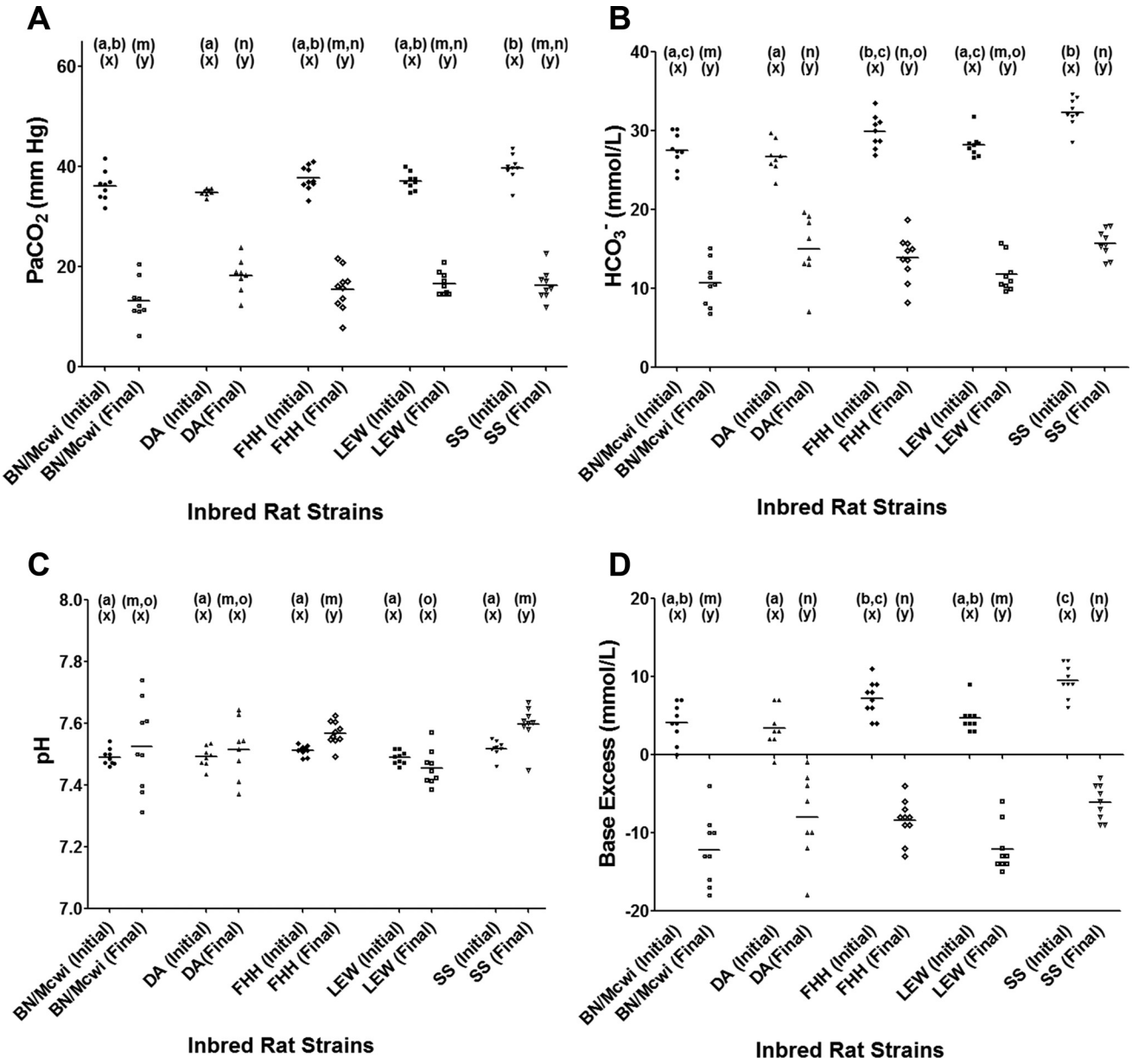

Fig. 1. Scatter plots of arterial blood measures depicting initial and final values for each rat. *A:* Pa_{CO_2}; *B:* HCO_3^-; *C:* pH; *D:* base excess. The mean for each strain and measure is represented by a solid horizontal line. Data with letters in common are not different ($P > 0.05$). Significant differences among initial vs. final comparisons within a strain are represented by *x* and *y*; comparisons of initial values among strains are represented by *a, b, c, d*; comparisons of final values among strains are represented by *m, n, o*. For clarity in presentation and to legibly visualize the scatter of the measures we have included only 4 panels per figure, wherein each panel represents one ABM. BN/Mcwi, Brown Norway Medical College of Wisconsin; DA, Dark Agouti; FHH, Fawn Hooded Hypertensive; LEW, Lewis; SS, Dahl Salt-Sensitive.

Intraclass correlation coefficients. Low intraclass correlation coefficients ($r_I < 0.5$) were observed for most ABM (both initial and final measures), suggesting that more variability (>50%) for each ABM exists within rather than among inbred rat strains (Table 3). In some cases (e.g., O_2 content, K^+), the predominant source of variation (within vs. among) changed by the end of hemorrhage as indicated by the transition of r_I across the threshold of 0.5 (Table 3).

Correlation of ABM to STaH

Because we were interested in measuring associations between the above-noted ABM and previously reported STaH, correlation coefficients with STaH were calculated for initial ABM, final ABM, and ΔABM, respectively, both across and within inbred rat strains. When one uses the criteria listed in MATERIALS AND METHODS, there were no relevant correlations between STaH and AMB or ΔABM across all five inbred rat strains. However, within BN/Mcwi and FHH strains, some potentially biologically relevant correlations were observed (Table 4).

DISCUSSION

Physiological differences have been recorded between humans who survive or die after surgery or trauma (59, 61, 63), and between outbred rats that survive or die after severe

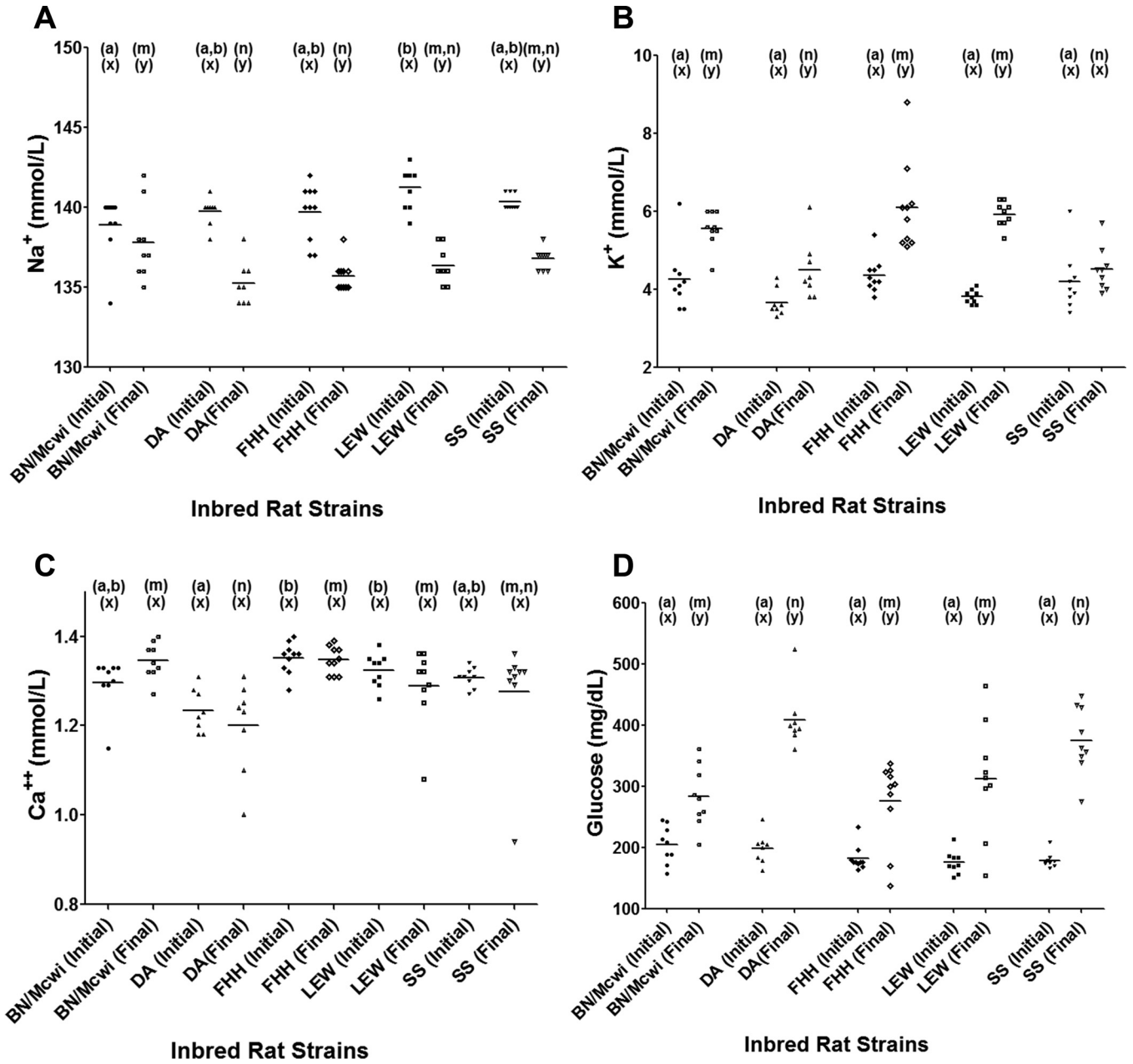

Fig. 2. Scatter plots of arterial blood measures depicting initial and final values for each rat. *A:* Na^+; *B:* K^+; *C:* Ca^{++}; *D:* glucose. The mean for each strain and measure is represented by a solid horizontal line. Data with letters in common are not different ($P > 0.05$). Significant differences among initial vs. final comparisons within a strain are represented by *x* and *y;* comparisons of initial values among strains are represented by *a, b, c, d;* comparisons of final values among strains are represented by *m, n, o.* For clarity in presentation and to legibly visualize the scatter of the measures, we have included only 4 panels per figure wherein each panel represents one ABM.

hemorrhage (67–68). Here we attempt to cautiously interpret differences in some early systemic blood measures during hemorrhage among inbred rat strains. It is worth reminding readers that in the current model, the hemorrhaged rats were conscious, breathing naturally, and were not administered resuscitation fluid (34). Therefore, rats and associated ABM were uninfluenced by potentially confounding effects of anesthetics, artificial respiration, or exogenous fluids. However, the ABM could, potentially, have been influenced in a strain-dependent manner by transcapillary refill that is known to occur during hemorrhage and shock (17, 43), but this mechanism was not investigated in the present study. ABM were measured at the beginning and end of a 26-min 47% hemorrhage, and hence represent normovolemic values and early hypovolemic shock measurements, respectively. These ABM data are the first such recordings for these inbred rat strains that differ in STaH. Further, although many studies have reported intrainbred strain variation for various phenotypes, this is the first study to quantify and statistically compare the intrastrain variation relative to the interstrain variation.

Severe hemorrhage results in reduced blood volume, blood pressure, and cardiac output leading to diminished tissue per-

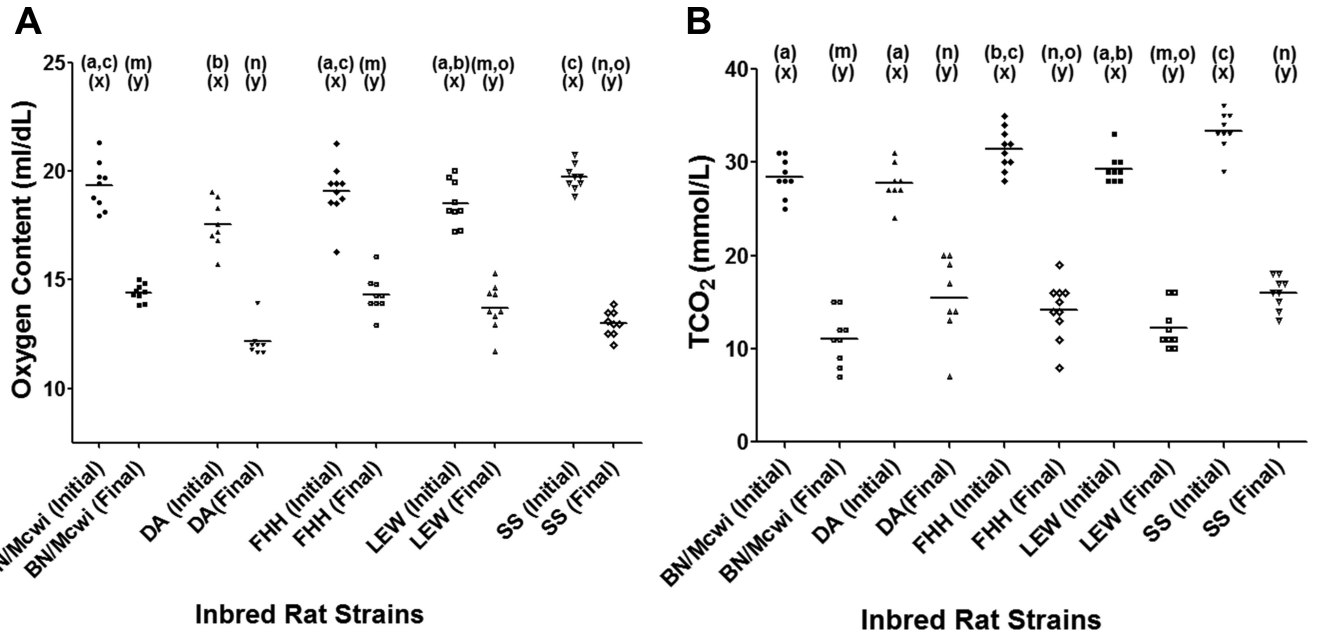

Fig. 3. Scatter plots of arterial blood measures depicting initial and final values for each rat. *A:* oxygen content; *B:* TCO_2. The mean for each strain and measure is represented by a solid horizontal line. Data with letters in common are not different ($P > 0.05$). Significant differences among initial vs. final comparisons within a strain are represented by *x* and *y;* comparisons of initial values among strains are represented by *a, b, c, d;* comparisons of final values among strains are represented by *m, n, o.* For clarity in presentation and to legibly visualize the scatter of the measures, we have included only 4 panels per figure wherein each panel represents one ABM.

fusion and O_2 delivery (24, 67, 69). Subsequently, there is an increase in anaerobic metabolism that raises lactate and decreases HCO_3^-, thereby resulting in metabolic acidosis and reduced pH (69). Oxygen delivery (DO_2) to tissues is determined as the product of cardiac output and oxygen content (25). Surprisingly, both initial and final oxygen content were lower in the strain with the longest STaH (the DA) compared with other strains. Because cardiac output was not measured, it remains a matter of speculation whether the reduced oxygen content resulted in reduced DO_2.

As DO_2 decreases, cells experiencing hypoxia switch to anaerobic glycolysis as reflected by increases in blood lactate concentrations after severe hemorrhage (67–68). In our study, we were unable to measure blood lactate concentrations, but BE was measured. Although, BE was not correlated with STaH, it remained nearly constant (least change) during hemorrhage in DA rats. Earlier reports on hemorrhaged rodents also found higher BE in survivors vs. nonsurvivors (32, 67). The calculated BE reflects the combined effects of blood bicarbonate and H^+ concentrations. A decrease in BE is closely associated with increases in oxygen debt (19, 53–54), and has been used as a predictor of mortality after hemorrhage (14, 36, 54). It is possible that the small change in BE during hemorrhage among DA rats is associated with minimal increase in oxygen debt (not measured in the current study), resulting in a better survival mechanism.

Bicarbonate ions (HCO_3^-) constitute one of the most important buffers in blood (51), and their concentrations are closely related to CO_2 in blood. Together, Pa_{CO_2} and HCO_3^- are used to calculate TCO_2. Indeed, HCO_3^- and TCO_2 decreased in parallel with Pa_{CO_2}, and as with Pa_{CO_2}, the smallest decrease occurred in the longest-lived strain, DA rats. To-

Table 1. *Changes in arterial blood measures among inbred rat strains during a 47% hemorrhage*

Variable	Inbred Rat Strain				
	BN/Mcwi (n = 9)	DA (n = 8)	FHH (n = 10)	LEW (n = 9)	SS (n = 9)
ΔPa_{CO_2} (mmHg)	-22.9 ± 1.7^a	-16.7 ± 1.1^b	-22.2 ± 0.9^a	-20.5 ± 0.6^a	-23.5 ± 0.9^a
ΔHCO_3^- (mmol/liter)	-16.9 ± 1.2^a	-11.7 ± 1.3^b	-16.3 ± 0.6^a	-16.5 ± 0.5^a	-16.6 ± 0.5^a
ΔpH	0.03 ± 0.05^a	0.02 ± 0.03^a	0.06 ± 0.01^a	-0.03 ± 0.02^a	0.07 ± 0.02^a
ΔBE (mmol/liter)	-16.3 ± 1.7^a	-11.4 ± 1.7^b	-15.6 ± 0.6^a	-16.8 ± 0.7^a	-15.6 ± 0.7^a
ΔNa^+ (mmol/liter)	-1.11 ± 0.75^a	-4.5 ± 0.5^b	-4 ± 0.49^b	-4.89 ± 0.42^b	-3.56 ± 0.24^b
ΔK^+ (mmol/liter)	$1.3 \pm 0.27^{a,b,c}$	$0.8 \pm 0.2^{b,c}$	$1.7 \pm 0.4^{a,b}$	2.1 ± 0.1^a	0.3 ± 0.27^c
ΔGlucose (mg/dl)	78.3 ± 15.58^a	210 ± 10.3^b	94 ± 25.5^a	$136 \pm 30^{a,b}$	197 ± 20^b
ΔOxygen content (ml/dl)	-4.9 ± 0.4^b	-5.4 ± 0.3^b	-4.8 ± 0.3^b	-4.8 ± 0.2^b	-6.7 ± 0.3^a
ΔTCO_2 (mmol/liter)	-17.3 ± 1.2^a	-12.2 ± 1.3^b	-17.2 ± 0.7^a	-17.1 ± 0.6^a	-17.3 ± 0.5^a
ΔCa^{2+} (mmol/liter)	0.05 ± 0.02^a	-0.03 ± 0.03^a	-0.005 ± 0.01^a	-0.04 ± 0.02^a	-0.031 ± 0.04^a

Values represent the change (Δ) in each measure (i.e., final value minus initial value) for each inbred rat strain (mean ± SE). For each measure, strains that share a common superscript are not different ($P > 0.05$) as determined by the Student-Newman-Keul's test. BN/Mcwi, Brown Norway/Medical College of Wisconsin; DA, Dark Agouti; FHH, Fawn Hooded Hypertensive; LEW, Lewis; SS, Dahl Salt-Sensitive; Pa_{CO_2}, partial pressure of arterial carbon dioxide; HCO_3^-, bicarbonate; BE, base excess; TCO_2, total carbon dioxide.

Table 2. *Coefficients of variation of initial final arterial blood measures and their comparison*

	$PaCO_2$	HCO_3^-	pH	BE	Na^+	K^+	Glucose	O_2 Content	TCO_2	Ca^{2+}
Initial										
BN/Mcwi	8.14	7.9	0.35	60.13†	1.41	18.98†	15.0†	5.66	7.27	4.44
DA	2.03	7.6	0.44	79.08†	0.63	9.56	12.64†	6.37	7.64	3.92
FHH	6.46	7.02	0.2	31.26†	1.26	10.09†	11.02†	6.65	7.07	2.58
LEW	4.55	5.41	0.27	38.63†	0.92	4.62	10.61†	5.44	5.39	2.8
SS	6.69	5.73	0.34	21.89†	0.35	18.25†	6.96	2.96	6.18	1.7
P	<0.05†	>0.75	>0.25	<0.025†	<0.01†	<0.005†	>0.25	>0.25	>0.75	>0.05
Final										
BN/Mcwi	31.85*†	26.99*†	1.94*	36.54†	1.69	8.49*	17.57†	2.74	25.23†	3.04
DA	18.93*†	27.97*†	1.27*	69.76†	1.03	16.91†	12.09†	6.09	28.64†	8.54
FHH	26.79*†	21.15*†	0.51*	31.35†	0.70	18.68†	24.78*†	6.03	21.46†	2.24
LEW	13.89*†	19.07*†	0.77*	25.26†	0.82	5.52	29.96*†	7.74	19.1†	6.71
SS	18.66*†	11.21†	0.83*	36.99†	0.49	12.41†	14.63*†	4.45	10.82†	10.0†
P	>0.1	>0.1	<0.001†	>0.05	<0.005†	<0.025†	>0.05	>0.05	>0.1	<0.05†

Initial refers to blood measures recorded at the beginning of hemorrhage (first 0.5 ml); final refers to blood measures recorded at the end (last 0.5 ml) of the 26-min, 47% controlled hemorrhage. $PaCO_2$, partial pressure of arterial carbon dioxide; HCO_3^-, bicarbonate; BE, base excess; TCO_2, total carbon dioxide; BN/Mcwi, Brown Norway/Medical College of Wisconsin; DA, Dark Agouti; FHH, Fawn Hooded Hypertensive; LEW, Lewis; SS, Dahl Salt-Sensitive. *Indicates that final coefficient of variation (CV) is different ($P < 0.05$) than initial CV for that measure and inbred rat strain. †Indicates CV >10% and significant *P* value (differences in CVs among strains for each measure).

gether, such data (oxygen content, HCO_3^-, BE) might indicate that one potential mechanism by which DA have improved survival is their ability to better maintain an appropriate acid-base balance during a global ischemic challenge.

Contrary to the increased P_{CO_2} expected to occur at the tissue level and in venous blood during severe hemorrhage (40, 57, 69–70), Pa_{CO_2} decreases during the course of hemorrhage as observed in the current and other studies (28–29, 67–69). This decrease in Pa_{CO_2} is probably due to hyperventilation that occurs as a respiratory compensatory mechanism to neutralize metabolic acidosis. The longest-lived strain, DA, with the least change in BE, also had the least change in Pa_{CO_2} and the highest final Pa_{CO_2} concentrations. Although no definitive cause-and-effect relationship can be ascribed here, higher arterial Pa_{CO_2} has been shown to be associated with improved survival in hemorrhaged animals (32, 67). Concordantly, in the BN/Mcwi rat, which had shorter STaH and lower Pa_{CO_2} compared with the DA rat, the final Pa_{CO_2} was directly correlated with STaH, whereas the ΔPa_{CO_2} was inversely correlated with STaH.

In this study, arterial pH was remarkably stable at the time intervals measured. The SS strain was an exception, wherein pH increased by the end of hemorrhage. Such transient increases in pH have been reported (1, 30, 71). Other reports have shown arterial pH to numerically decrease in rats (68) and hamsters (32) early during hemorrhage (~30 min from start of hemorrhage).

Hemorrhage-induced metabolic acidosis is reported to affect K^+ homeostasis and increase blood K^+ concentrations (67), which may result in death (68). Each inbred rat strain (except SS) demonstrated the expected increase in K^+ associated with severe hemorrhage (48, 55, 67–68), and in two inbred rat strains (BN/Mcwi and FHH) the increases in K^+ during hemorrhage were correlated with reduced survival time. Rapidly occurring hyperkalemia can also have adverse effects on cardiovascular and neuromuscular function (42, 58, 66). Hence, at least in some inbred rat strains such as BN/Mcwi, which showed an inverse correlation between ΔK^+ and STaH, cellular mechanisms [e.g., ATP-sensitive K^+ channels, inhibition of the Na^+-K^+ pump, shrinkage of the interstitial space (13, 73)] associated with maintaining cellular and extracellular K^+ levels might be involved in differential survival to hemorrhage. Na^+-K^+-ATPase activity is known to be altered in ischemic conditions resulting in either enhanced efflux of K^+ across the cell membrane or reduced influx of K^+. Ultimately, there is reduction of intracellular K^+ resulting in apoptosis (56, 76). Perhaps more remarkable and worthy of further investigation is the extremely modest and statistically insignificant increase in K^+ in SS rats, which also had higher pH after this very severe hemorrhage.

Although blood K^+ increased by the end of hemorrhage in most inbred rat strains, blood Na^+ decreased, as was previously noted in some studies (8, 15, 27, 67) but not others (10, 12). Among the electrolytes, calcium did not change during hemorrhage. Ionized calcium has multiple functions in the body and alterations in calcium level—both hypocalcemia and hypercalcemia—are associated with increased mortality in critically ill patients (20). However, no early differences in calcium levels were observed between survivors and nonsurvivors among both rats (67) and humans (31). In one study, differences in calcium levels were noted after 3 days of trauma (72). Concordantly, in the current study, there is no difference in

Table 3. *Calculated intraclass correlation coefficients for different blood measures*

Blood measure	pH	Pa_{CO_2}	O_2 Content	Na^+	K^+	Glucose	HCO_3^-	BE	TCO_2	Ca^{2+}
Initial measures	0.166	0.339	0.329	0.208	0.146	0.181	0.543	0.524	0.533	0.492
Final measures	0.217	0.133	0.582	0.269	0.504	0.396	0.302	0.291	0.276	0.274

Intraclass correlation coefficients, r_I, represent the proportion of the total variation that exists among inbred rat strains. A higher r_I (>0.5) indicates that more of the observed variation is between strains than within strains. Conversely, a lower r_I (<0.5) indicates more of the observed variation is within strains than between strains. Pa_{CO_2}, partial pressure of arterial carbon dioxide; HCO_3^-, bicarbonate; BE, base excess; TCO_2, total carbon dioxide.

Table 4. *Strain-specific correlations between survival time after hemorrhage and arterial blood measures*

Variable	Inbred Rat Strain	Correlation Coefficient (r)	Probability
ΔPa_{CO_2} (mmHg)	BN/Mcwi	−0.76	0.02
Final Pa_{CO_2}	BN/Mcwi	0.89	0.0001
ΔK^+ (mmol/l)	BN/Mcwi	−0.84	0.005
ΔK^+ (mmol/l)	FHH	−0.80	0.006
ΔGlucose (mg/dl)	FHH	0.73	0.020
Final glucose	FHH	0.70	0.024
ΔOxygen content (ml %)	FHH	0.74	0.02

Presented are only those correlations (r) that are significant ($P < 0.05$), have minimal scatter about the least- squares regression line, do not have outliers that might influence r, and that have a coefficient of determination (r^2) ≥0.5 (i.e., ≥50% of the variance in one variable can be explained by the variance in the second variable). Pa_{CO_2}, partial pressure of arterial carbon dioxide; BN/Mcwi, Brown Norway/Medical College of Wisconsin; FHH, Fawn Hooded Hypertensive.

ionized calcium between the normovolemic and hypovolemic measurements of any strain.

Transient hyperglycemia during early hemorrhagic shock is a long-known phenomenon (4, 9, 18, 68) and results from a number of possible mechanisms (46, 48, 52). In more prolonged hemorrhagic shock, however, blood glucose concentrations decrease below the normal range (39, 46, 68), commensurate with diminished liver glycogen and altered hepatocyte calcium homeostasis (39). Such changes are associated with the onset of irreversible hemorrhagic shock. The ability to maintain higher blood glucose levels after severe hemorrhage appears to be associated with better early survival (65, 68). However, in trauma patients, early hyperglycemia appears to be associated with poor survival and more infections post trauma (37). In addition to contributions to energy production, hyperglycemia in the early phase of hemorrhage may contribute to an increase in plasma osmolality, which along with decreased capillary hydrostatic pressure, promotes fluid absorption and thus plasma volume expansion and refill (21, 47). In this study we observed the highest early hyperglycemic response to hemorrhage in the DA strain, with the highest STaH.

Of special interest is that in addition to the interstrain differences, there were remarkable differences for most ABM within each inbred strain as well. The observed variability of ABM may result from multiple sources: *1)* possible technical error; *2)* diurnal variations in ABM; *3)* seasonal variations in ABM; *4)* age- and gender-associated variations in ABM; *5)* environmental differences among strains at respective vendors; *6)* potential environmental differences within our vivarium; or *7)* biological variation that can be subclassified into *a)* random differences among rats, *b)* genetic effects, and *c)* epigenetic effects. As evidence that sources *1)* through *6)* each generate minimal to negligible amounts of variation, we note the following six points. *1)* A single instrument with one operator determined all ABM throughout the study, and the variation associated with standard samples is extremely small. *2)* Blood samples were collected and ABM were measured during the same 4-h period each day. *3)* The entire study was completed in ~3 mo. *4)* All rats were males and experimental procedures were conducted on them at approximately the same age. *5)* Minor environmental variations could have existed between different vendors. However, an approximate 3-wk acclimatization period with identical laboratory conditions was provided prior to experimentation for each rat. Further, the within-strain variation observed clearly argues against vendor-based environmental differences. *6)* Variables such as temperature, humidity, light-dark cycle, bedding, food-water source, and supply were monitored routinely and consistency of these was maintained.

As suggested earlier for STaH (34), inbred rats of each strain are genetically similar (2). Hence, with consistent environmental conditions (50), between-inbred strain differences may primarily be due to genetic differences among inbred strains. Differences in ABM among inbred rat strains were, therefore, expected. The wide range of intrastrain ABM measurements evidenced by high CVs may be within the normal biological range of outbred rats. However, considering the genetic and environmental similarities between rats of a given inbred strain, the wide range of ABM (i.e., high CVs) was unexpected. Moreover, for some measures (such as initial K^+), the coefficients of variation differed among inbred rat strains, suggesting greater intrastrain variation for certain measures in some inbred rat strains. Within each strain, the differences in CVs between initial and final recordings of ABM were also remarkable and indicate the individual-specific changes occurring while transitioning to the hypovolemic state.

As further evidence of the intrastrain variation, the calculated low intraclass correlation coefficients, r_I, emphasized the presence of high within-strain variation, and indicated greater intrastrain variation than interstrain variation for most ABM. The intraclass correlation coefficient has been shown to be useful as a measure of technical replicates and biological replicates for laboratory experiments (49). It has been suggested that if the intraclass correlation coefficient (r_I) is ≥0.5, it is usually optimal to obtain no more than two replicates per individual (7). Because animals of an inbred strain are genetically similar and have been raised in similar environmental conditions, measurements for each ABM from rats of an inbred strain should be comparable to biological replicates. This 0.5 value is therefore suggestive of a cut-off r_I value. Thus, 12 of 16 ABM (initial and final) had <0.5 r_I values (Table 3).

Within-strain variation in inbred animals for various phenotypes have been reported (5, 22, 26, 38, 74, 78), but not quantified in this manner. Some within-strain variation may be accounted for by residual genetic heterozygosity, sporadic de novo polymorphisms, and random biological variation among individual rats. However, on the basis of the size of the variation, we hypothesize that the variation may be at the least partly due to individual-specific epigenetic processes (such as DNA methylation). Such processes have been postulated to be responsible for phenotypic differences among monozygotic human twins and inbred animals (23, 75). It is also possible that such individual-specific epigenetic processes may be responsible for some of the interstrain variation observed.

Three limitations of this study need to be considered. First, the 24-h recovery period between surgery and hemorrhage that was given to circumvent possible confounding effects of anesthetics and analgesics. Consequently, potential alterations in food and water intake postsurgery became a part of the model. Such alterations may result in some baseline changes in a strain-dependent manner. As expected, strain-dependent differences in food and water consumption were observed after

surgery. However, there were no correlations between either food or water consumed after surgery and STaH or ABM measures. Interestingly, although DA rats had the most drastic decrease (approximately fourfold) in food consumption after surgery (compared with food consumption on the day before surgery), their initial glucose levels were comparable to other strains. Second, ABM were measured at just two time points (i.e., initial and final). This occurred because the study was primarily aimed at QTL/gene identification and we believed that two measures of ABM were sufficient for investigating a preliminary association of these measures with survival time in these inbred rat strains. These data, besides providing novel information about ABM and STaH in these inbred strains, complement data from other similar studies (including other rat strains and other species) that have included multiple time points (67–68). Third, all instrument-based measurements of blood samples were performed at 37°C (i-STAT heats the samples to 37°C before analysis). However, body temperature was not measured in these conscious, unrestrained rats. Because hypothermia is a common feature associated with hypovolemic shock, it is possible that body temperature decreased during hemorrhage in a strain-dependent manner and influenced some actual (i.e., in vivo), final ABM measures.

In conclusion, there are measurable differences in arterial blood indices (in both the normovolemic and hypovolemic states) among inbred rat strains that provide some physiological clues concerning cellular mechanisms associated with differences in STaH for these inbred rat strains, and suggest a genetic basis for such variations. Surprisingly, besides these interstrain differences, there was large intrastrain variation in many measures as well. The enormity of the intrastrain variation and evaluation of the various sources potentially responsible for such variation suggest that epigenetic processes such as DNA methylation could be a factor responsible for intrastrain variation in STaH and ABM.

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DISCLAIMER

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.R., B.S.K., and H.G.K. conception and design of research; R.R. and H.G.K. performed experiments; R.R. and H.G.K. analyzed data; R.R., B.S.K., and H.G.K. interpreted results of experiments; R.R. and H.G.K. prepared figures; R.R. drafted manuscript; B.S.K. and H.G.K. edited and revised manuscript; H.G.K. approved final version of manuscript.

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